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Review

Potential of South African medicinal plants used as anthelmintics – Their efficacy, safety concerns and reappraisal of current screening methods

A.O. Aremu, J.F. Finnie, J. Van Staden *

Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

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Abstract

Studies on anthelmintic activity of medicinal plants have received insufficient interest and attention from researchers despite the high incidence of helminth infections in the poorer communities of South Africa. There are only a few anthelmintic remedies available which are inadequate in terms of accessibility, affordability and probably efficacy. In this review, we reappraised the various anthelmintic studies on South African medicinal plants to highlight how much and/or how little is known. The rich botanical and medicinal plant knowledge in South Africa is an indication of the potential of discovering potent treatments against helminth diseases in both humans and livestock. A total of 115 plant species encompassing 43 families screened for their anthelmintic potential (mainly nematodes) are listed in the current review. Combretaceae and Fabaceae were the most commonly used families. *Tetradenia riparia*, *Hypoxis colchicifolia*, *Apodytes dimiata* and *Leucosidea sericea* are a few examples of the South African species that have demonstrated promising anthelmintic activity. Even though other species such as *Dicerocaryum eriocarpum*, *Berchemia zeyheri* and *Acorus calamus* are potent anthelmintics, caution must be exercised in administering these plant extracts because of their potential toxic effects. Besides the benefit of validating the efficacy of traditional medicines, compounds from South African medicinal plants could also provide a template for novel synthetic anthelmintics. However, the major bottleneck in exploring more South African medicinal plants for anthelmintic properties is probably the lack of a robust, rapid and reliable screening technique as well as adequate funding schemes. Perhaps, there could be more success stories via increased research outputs with the development and availability of high throughput screening methods for assessing the activity of these medicinal plants as well as their resultant bioactive compounds. In addition, accessibility of funds to acquire latest technology and motivate researchers will inevitably stimulate more studies geared toward alleviating the problems posed by helminth infections.

Keywords: Antimicrobial; Ethnopharmacology; Neglected diseases; Pharmaceuticals; Toxicology; Traditional medicine

Contents

Abbreviations: AAD, amino-acetonitrile derivative; ADT, adult development test; AIDS, acquired immune-deficiency syndrome; CA, colorimetric assay; CFDA, 5(6)-carboxyfluorescein diacetate; DALYs, disability-adjusted life years; DBA, developmental and behavioral assay; EHT, egg hatch test; HIV, human immunodeficiency virus; INT, *p*-iodonitrotetrazolium chloride; LAMA, larval arrested morphology assay; LDA, larval development assay; LMA, larval motility assay; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; STH, soil-transmitted helminth; WHO, World Health Organization.

* Corresponding author. Tel.: +27 33 2605130; fax: +27 33 2605897.
E-mail address: rcpgd@ukzn.ac.za (J. Van Staden).

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1. Introduction

On a global level, Brooker et al. (2004) recommended the need for national governments to encourage more research into traditional medicinal plants and emphasized the importance of developing good policies, regulations and trade standards. Along this line, the South African government has proposed the integration of traditional medicine into the national healthcare system. However, the absence of stringent regulation mechanisms and secrecy within traditional medicine remains major stumbling blocks (Gqaleni et al., 2007). Jäger (2005) raised concern on the relatively limited research focusing on diseases such as diarrhea, schistosomiasis and other helminthiasis affecting the majority of the population who are the main users of traditional medicine. For instance, only a few research groups such as the Research Centre for Plant Growth and Development, University of KwaZulu-Natal and the Phytomedicine Programme, Department of Paraclinical Sciences, University of Pretoria, have been actively involved in anthelmintic research. Furthermore, the lack of regulation in the prescription and usages of traditional medicines as well as inadequate toxicological evidence on the safety of the medicinal plants remain a problem (Fennell et al., 2004; Verschaeve and Van Staden, 2008). Most importantly, there needs to be a holistic approach for traditional medicine that creates avenues to channel scientific information back to traditional healers (Jäger, 2005). Helminth infections of both humans and livestock are of considerable importance (Hoste and Torres-Acosta, 2011). Anthelmintics dominate the animal pharmaceutical industry being sold in the largest volume and having a high value (see International Federation for Animal Health website: http://www.ifahsec.org). Anthelmintic research and development has thus demanded a very large share of the animal health pharmaceutical development effort and is probably the only area of such research where efforts and success in animal health exceed those in human health (McKellar and Jackson, 2004).

In this review, we highlight the limited available scientific data on helminth diseases in both humans and livestock despite the significant problems associated with the infections. In addition, the various techniques and safety of the South African medicinal plants screened for their anthelmintic properties in recent years (up till January 2012) are assessed. The review is expected to serve as an impetus for more studies to explore the rich South African flora.

2. Helminth infections and associated problems

Helminth infections such as ascariasis, hookworm infection and schistosomiasis constitute the core 13 neglected tropical diseases (NTDs) affecting the world population (Hotez et al., 2007). Majority of the NTDs are well-pronounced among impoverished populations living in marginalized regions (Hotez et al., 2009). In most developing countries, helminth infections have remained a major health concern because factors that pre-dispose humans to these infections abound in these areas. These incapacitating diseases continue to inflict severe disability and often death. In terms of disability-adjusted life years (DALYs), Hotez et al. (2006) estimated the global burden of helminth infections to be 39 million life years which is comparable to tuberculosis (34.7 million DALYs) and malaria (46.5 million DALYs), the two major human infectious diseases associated with high mortality rates. As a result, helminthiases have generally become the indices of low levels of socio-economic status of the countries where the infections are prevalent (Hotez et al., 2009). Children, especially those less than five years old, have been identified as the most vulnerable group with high rates of helminth infection (Sinniah, 1984).

The infecting helminth species, degree of infection and host age, determine the clinical symptoms of helminth infection (Wang et al., 2008). A number of factors that determine the pathogenicity of helminth infections include: exposure and entry; adherence and replication; cell and tissue damage; disruption, invasion and inactivation of host defenses (Murray et al., 1998). Researchers have also observed a decline in host immune status as a result of helminth infection thereby increasing the host susceptibility to other pathogens and secondary infections (Borkow and Bentwich, 2006; Brooker et al., 2004). Increasing direct and indirect evidences has indicated that helminth infection create an opportunity for more rapid infection by the human immunodeficiency virus (HIV) as well as quicker progression to acquired immune-deficiency syndrome (AIDS) and tuberculosis. In addition, the efficacy of some vaccines against HIV may be impaired

by chronic helminth infection (Elias et al., 2006; Fincham et al., 2003; Wolday et al., 2002). Ironically, studies have indicated that ailments such as asthma, heart and Crohn's diseases could be suppressed by some groups of helminths (Elliott et al., 2007; Magen et al., 2005). Consequently, it has been predicted that the concept of some helminths may change from that of a strict parasite – harmful to the host, to a mutualist – providing benefit to the host as governors of immune-mediated inflammation in the near future (Elliott et al., 2007). Active research to support this theory is on-going but it is outside the scope of this review.

Due to the asymptomatic nature of helminth diseases, they can remain undetected and children born in an endemic region may harbor the worms for the greater parts of their lives (WHO, 1987). Majority of the helminths consume nutrients from their host, thereby causing or aggravating malnutrition which results in retarded growth and physical development. Consequently, symptoms like retarded cognitive development, iron deficiency anemia, abdominal pains and related health problems are characteristic features of most heavy helminth infections (Kirwan et al., 2009). Generally, helminth infections are regarded as a global problem of medical, educational and economic significances because of the long term chronic inflammatory disorders and disabling effects when left untreated (Prichard et al., 2012; Stepek et al., 2006; Wang et al., 2008).

2.1. Prevalence of helminth infections in South Africa

In South Africa, especially the rural areas, the prevalence rate of helminth infections is alarming because factors such as poor sanitation and malnutrition that pre-dispose humans to these infections abound. Although there is no official data on prevalence and intensity of helminth infections in the country as a whole (Fincham et al., 1996; Schutte et al., 1995), incidence of high prevalence rate of intestinal nematode infection in Mpumalanga (Evans et al., 1987), the Western Cape (Fincham et al., 1996) and KwaZulu-Natal (Appleton et al., 1999; Mabaso et al., 2004) have been reported. Of particular interest is KwaZulu-Natal, the third poorest province in South Africa, which has a high mortality and prevalence of HIV/AIDS (Hirschowitz and Orkin, 1997). In KwaZulu-Natal, the transmission and epidemiology problems of helminth infections such as *Ascaris lumbricoides* and *Trichuris trichiura* have remained relatively high over a long period of time (Jinabhai et al., 2001) and children remain the most vulnerable group (Saathoff et al., 2004).

As with helminth infection rates in humans (Mkhize-Kwitshana et al., 2011), the absence of prevalence surveys in recent times makes it difficult to substantiate the postulated high incidence rate in South Africa (Kaplan and Vidyashankar, 2012). As is common in the tropics, *Haemonchus contortus* and *Trichostrongylus colubriformis* remain the most common nematodes that hinder production of ruminants in South Africa (Horak and Ursula, 2004; Van Wyk et al., 1999). Helminthiasis is often controlled with the use of chemotherapeutics; however, the development of resistance to most of the commercially available anthelmintics has become a severe problem globally (Hoste and Torres-Acosta, 2011). In fact, the first case of anthelmintic resistance (against ivermectin) was reported in South Africa (Van Wyk et al., 1987).

3. Limitations of existing treatment regimes

3.1. Available classes of anthelmintic drugs and their mode of action

The existing pharmacopeia for both human and livestock helminth infections is severely limited (Geary et al., 2010). The control of helminth infections requires a multi-faceted approach due to the complex life cycles of the causative organisms as well as environmental influences (Hoste and Torres-Acosta, 2011; Whitfield, 1996). Currently, the use of broad spectrum chemotherapy is required for multiple worm infection in humans (Booth et al., 1998) and livestock (Hoste and Torres-Acosta, 2011). In addition, proper health education, good hygiene and improved sanitation are indispensable in alleviating the spread of the causative organisms (Fincham et al., 2003; Savioli et al., 1992; Stepek et al., 2006).

Based on their chemical structure and mode of action, there are only four classes of anthelmintics. The Class 1 drugs, known as the benzimidazoles (*e.g.* albendazole and mebendazole) were introduced in 1961. These drugs bind to free *beta*-tubulin and inhibit its polymerization, thereby interfering with the microtubule-dependent glucose uptake by the parasite (Rang et al., 2003). Class 2 imidazothiazole drugs *e.g.* levamisole, were discovered in 1966 and act by stimulating the nicotinic acetylcholine receptors leading to the paralysis of the worms which are subsequently washed out of the intestine by peristalsis (Stepek et al., 2006). The third class of drugs, generally referred to as macrocyclic lactones, were introduced in the early 1980s (Chabala et al., 1980). The first of such drugs was ivermectin which interferes with GABA-mediated neurotransmission resulting in paralysis and death of the helminth. These drugs act by paralysis of body-wall muscles in nematodes (Behnke et al., 2008). In 1997, moxidectin, an analogue of ivermectin, which is the most potent macrocyclic lactone, was developed due to incidences of resistance and ineffectiveness of ivermectin against some groups of helminths (Geary, 2005). The limited potency and safety of the three classes necessitated the search for new anthelmintics (Whitfield, 1996). Finally in 2009, Novartis launched Zolvix®, a new class of anthelmintic referred to as the amino-acetonitrile derivatives (AADs) after 28 years of intensive research (Kaminsky et al., 2008b). The AADs interfere with a unique ACR-23 nicotinic acetylcholine receptor subunit leading to the paralysis of the worms. In addition, AADs are relatively safe as evident from their low toxicity to mammals after various tests (Ducray et al., 2008; Kaminsky et al., 2008a). Further studies by researchers involve the possibility of the potential synergetic action of different drugs (Tritten et al., 2012a,c). In view of the wide diversity of helminthiasis and scope of the current review, more details on available chemotherapeutics, especially against other group of helminths such as cestodes and trematodes are available elsewhere (Geary et al., 2010).

3.2. Rationale for exploring alternative treatments

'Global worming' is evident in countries such as South Africa, United States, Brazil and New Zealand (Kaplan and Vidyashankar,

Table 1
Examples of South African medicinal plants screened for their anthelmintic potential.

Family Plant species	[a]Plant part(s)	[b]Assay method	Test organism(s)	[c]Concentration (mg/ml)	Safety findings? Method(s) and reference	Reference
Alliaceae (Liliaceae)						
Tulbaghia violacea Harv.	Tb	DBA – *in vitro*	*Caenorhabditis elegans*	1.00	Negative – Ames and VITOTOX® (Elgorashi et al., 2003; Reid et al., 2006); and positive – micronucleus test (Taylor et al., 2003)	McGaw et al. (2000)
Amaranthaceae						
Amaranthus hybridus L.	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – brine shrimp toxicity assay (McGaw and Eloff, 2005)	McGaw and Eloff (2005)
Anacardiaceae						
Rhus geinzii Sond	R	DBA – *in vitro*	*Schistosoma haematobium*	50.0	Negative – Ames and VITOTOX® (Elgorashi et al., 2003)	Sparg et al. (2000)
Rhus lancea L.f.	Bk L	DBA – *in vitro*	*C. elegans*	1.00 0.50	Positive – brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2007)
Sclerocarya birrea (A. Rich.) Hochst.	R	DBA – *in vitro*	*S. haematobium*	25.0	Negative – Ames and VITOTOX® (Elgorashi et al., 2003); and brine shrimp toxicity assay (McGaw et al., 2007)	Sparg et al. (2000)
Sclerocarya birrea (A. Rich.) Hochst.	Bk	DBA – *in vitro*	*C. elegans*	0.50	Negative – Ames and VITOTOX® (Elgorashi et al., 2003); and brine shrimp lethality/toxicity (McGaw et al., 2007)	McGaw et al. (2007)
Apiaceae						
Hetermorpha trifoliata (Spreng.) Cham. & Schltdl.	L	DBA – *in vitro*	*C. elegans*	2.00	Negative – Ames and VITOTOX® (Elgorashi et al., 2003); and positive – micronucleus test and comet assay (Taylor et al., 2003)	McGaw et al. (2000)
Pimpinella caffra (Eckl. & Zeyh.) D. Dietr.	Wp	DBA – *in vitro*	*C. elegans*	1.00	Unknown	McGaw et al. (2000)
Apocynaceae						
Acokanthera oblongifolia (Hochst.) Codd	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – Ames and VITOTOX® (Elgorashi et al., 2003)	McGaw et al. (2000)
Acokanthera oppositifolia (Lam.) Codd	L T	CA – *in vitro*	*C. elegans*	0.52 2.08	Unknown	Aremu et al. (2010b)
Asclepias fruticosa L.	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – brine shrimp toxicity assay (McGaw and Eloff, 2005)	McGaw and Eloff (2005)
Landolphia kirkii Dyer ex Hook. f.	–	DBA – *in vitro*	*S. haematobium*	25.0		Sparg et al. (2000)
Mondia whitei (Hook. f.) Skeels	R	DBA – *in vitro*	*S. haematobium*	25.0	Unknown	Sparg et al. (2000)
Secamone filiformis (L.f.) J.H. Ross.	Ap	DBA – *in vitro*	*C. elegans*	n/a	Negative – brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2007)
Thevetia peruviana (Pers.) K. Schum	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – brine shrimp toxicity assay (McGaw and Eloff, 2005)	McGaw and Eloff (2005)
Araceae						
Acorus calamus L.	R+Rm	DBA – *in vitro*	*C. elegans*	1.00	Positive – brine shrimp toxicity assay (Padmaja et al., 2002)	McGaw et al. (2000)
Araliaceae						
Cussonia spicata Thunb.	R	DBA – *in vitro*	*C. elegans*	n/a	Positive – brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2007)
Asteraceae						
Artemisia afra Jacq. ex Willd.	L	DBA – *in vitro*	*C. elegans*	1.00	Negative – Ames and VITOTOX® (Elgorashi et al., 2003); and micronucleus test (Taylor et al., 2003)	McGaw et al. (2000)
Berkheya speciosa (DC.) O. Hoffm.	R	DBA – *in vitro*	*S. haematobium*	3.13	Unknown	Sparg et al. (2000)
Brachylaena discolor DC.	L	DBA – *in vitro*	*C. elegans*	1.00	Negative – toxicity to hepatocytes (Chang liver) and adipocytes (3T3–L1) cells (Van de Venter et al., 2008)	McGaw et al. (2000)
Felicia erigeroides DC.	L S	CA – *in vitro*	*C. elegans*	0.52 2.08	Unknown	Aremu et al. (2010b)
Hertia pallens (DC.) Kuntze	L	DBA – *in vitro*	*C. elegans*	1.00	Positive – brine shrimp toxicity assay (McGaw and Eloff, 2005)	McGaw and Eloff (2005)
Schkuhria pinnata (Lam.) Kuntze ex Thell.	Ap	DBA – *in vitro*	*C. elegans*	1.00	Positive – brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2007)

(continued on next page)

Table 1 (*continued*)

Family Plant species	[a]Plant part(s)	[b]Assay method	Test organism(s)	[c]Concentration (mg/ml)	Safety findings? Method(s) and reference	Reference
Vernonia colorata (Willd.) Drake	R	DBA – *in vitro*	*S. haematobium*	50.0	Negative – Ames and VITOTOX® (Elgorashi et al., 2003); and positive (toxic) – micronucleus test and comet assay (Taylor et al., 2003)	Sparg et al. (2000)
Bignoniaceae						
Kigelia africana (Lam.) Benth.	L	DBA – *in vitro*	*C. elegans*	1.00	Negative – Ames and VITOTOX® (Elgorashi et al., 2003)	McGaw et al. (2000)
Markhamia obtusifolia (Baker) Sprague.	L	DBA – *in vitro*	*Trichostrongylus colubriformis*	0.46 (EC_{50})	Negative – Vero monkey kidney cell line cytotoxicity assay (Nchu et al., 2011)	Nchu et al. (2011)
Cactaceae						
Cereus jamacaru DC.	Ap	DBA – *in vivo*	*Haemonchus contortus* *T. colubriformis*	n/a	Negative – toxicity to hematological and biochemical parameters (Messias et al., 2010)	Vatta et al. (2011)
Combretaceae						
Combretum apiculatum Sond.	L	DBA – *in vitro*	*C. elegans*	0.5	Positive – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	n/a		
Combretum bracteosum (Hochst.) Brandis	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	n/a		
Combretum celastroides Welw. ex M.A. Lawson	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	n/a		
Combretum collinum Fresen.	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	n/a		
Combretum edwardsii Exell	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	n/a		
Combretum erythrophyllum (Burch.) Sond.	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	n/a		
Combretum hereroense Schinz	L	DBA – *in vitro*	*C. elegans*	n/a	Positive – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	n/a		
Combretum imberbe Wawra	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	12.5		
Combretum imberbe Wawra	R	DBA – *in vitro*	*S. haematobium*	12.5	Negative – biochemical induction assay (McGaw et al., 2001)	Sparg et al. (2000)
Combretum kraussii Hochst.	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	12.5		
Combretum microphyllum Klotzsch	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	n/a		
Combretum mkhzense J.D. Carr & Retief	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	n/a		
Combretum moggii Exell	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	n/a		
Combretum molle R.Br. ex G. Don	L	DBA – *in vitro*	*H. contortus*	0.066 (LD_{50})	Negative – biochemical induction assay (McGaw et al., 2001)	Ademola and Eloff (2010)
Combretum molle R.Br. ex G. Don	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	25		
Combretum mossambicense (Klotzsch) Engl.	L	DBA – *in vitro*	*C. elegans*	n/a	Positive – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	n/a		
Combretum nelsonii Duemmer	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	12.5		
Combretum padoides Engl. & Diels	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	n/a		
Combretum paniculatum Vent.	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	25		
Combretum petrophilum Retief	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	25		
Combretum woodii Duemmer	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	n/a		
Combretum zeyheri Sond.	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – biochemical induction assay (McGaw et al., 2001)	McGaw et al. (2001)
			S. haematobium	n/a		
Cornaceae						
Curtisia dentate (Burm.f.) C.A.Sm.	L	DBA – *in vitro*	*C. elegans*	0.160	Unknown	Shai et al. (2009)
			H. contortus	0.310		
			T. colubriformis	0.160		

Table 1 (*continued*)

Family Plant species	[a]Plant part(s)	[b]Assay method	Test organism(s)	[c]Concentration (mg/ml)	Safety findings? Method(s) and reference	Reference
Crassulaceae						
Cotyledon orbiculata L. var. *dactylopsis*	L S	CA – *in vitro*	*C. elegans*	0.26 4.17	Negative – acute mammalian toxicity test (Amabeoku and Kabatende, 2012)	Aremu et al. (2010b)
Cotyledon orbiculata L. var. *orbiculata*	L S	CA – *in vitro*	*C. elegans*	0.26 4.17	Negative – acute mammalian toxicity test (Amabeoku and Kabatende, 2012)	Aremu et al. (2010b)
Crassula multicava Lemaire.	Wp	CA – *in vitro*	*C. elegans*	0.008	Negative – Ames test (Okem et al., 2012)	Okem et al. (2012)
Cyatheaceae						
Cyathea dregei Kunze	L R	CA – *in vitro*	*C. elegans*	0.52 2.08	Unknown	Aremu et al. (2010b)
Dracaenaceae (Liliaceae)						
Sansevieria hyacinthoides (L.) Druce	L	DBA – *in vitro*	*C. elegans*	1.00	Unknown	McGaw et al. (2000)
Ebenaceae						
Euclea divinorum Hiern.	Bk	DBA – *in vitro*	*C. elegans*	1.00	Positive (toxic) – Ames and VITOTOX® (Elgorashi et al., 2003)	McGaw et al. (2000)
Euclea divinorum Hiern.	R	DBA – *in vitro*	*S. haematobium*	25.0	Positive (toxic) – Ames and VITOTOX® (Elgorashi et al., 2003)	Sparg et al. (2000)
Euclea natalensis A.DC.	Bk	DBA – *in vitro*	*S. haematobium*	3.13	Positive (toxic) – Ames and VITOTOX® (Elgorashi et al., 2003); and negative – micronucleus and comet assay (Taylor et al., 2003)	Sparg et al. (2000)
Euphorbiaceae						
Antidesma venosum E. Mey. ex Tul.	R	DBA – *in vitro*	*S. haematobium*	12.5	Negative – Ames and VITOTOX® (Elgorashi et al., 2003). Positive – micronucleus test and comet assay (Taylor et al., 2003)	Sparg et al. (2000)
Jatropha multifida L.	L	DBA – *in vitro*	*C. elegans*	1.00	Negative – brine shrimp toxicity assay (McGaw and Eloff, 2005)	McGaw and Eloff (2005)
Ricinus communis L.	L	DBA – *in vitro*	*C. elegans*	1.00	Negative – Ames and VITOTOX® (Elgorashi et al., 2003); and positive – brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2000)
Ricinus communis L.	S+L	DBA – *in vitro*	*C. elegans*	1.00	Negative – Ames and VITOTOX® (Elgorashi et al., 2003); and positive – brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2007)
Ricinus communis L.	R	DBA – *in vitro*	*S. haematobium*	25.0	Positive – micronucleus test (Taylor et al., 2003)	Sparg et al. (2000)
Synadenium cupulare (Boiss.) L.C. Wheeler.	S+L	DBA – *in vitro*	*C. elegans*	n/a	Negative – brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2007)
Fabaceae						
Abrus precatorius L.	R	DBA – *in vitro*	*S. haematobium*	6.25	Unknown	Sparg et al. (2000)
Acacia karoo Hayne	–	DBA – *in vitro*	*S. haematobium*	50.0	Positive/negative – acute and sub-acute mammalian toxicity tests (Adedapo et al., 2008)	Sparg et al. (2000)
Afzelia quanzensis Welw.	R	DBA – *in vitro*	*S. haematobium*	6.25	Negative – Ames and VITOTOX®, (Elgorashi et al., 2003); and micronucleus test and comet assay (Taylor et al., 2003)	Sparg et al. (2000)
Albizia adianthifolia (Schumach.) W. Wight	L	DBA – *in vitro*	*C. elegans*	1.00	Unknown	McGaw et al. (2000)
Albizia versicolor Welw. ex Oliv.	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – brine shrimp toxicity assay (McGaw and Eloff, 2005)	McGaw and Eloff (2005)
Elephantorrhiza elephantina (Burch.) Skeels	R	DBA – *in vivo*	*Strongyle* *Trichuris* spp. *Eimeria* spp.	500 mg/kg	Negative – acute, sub-acute and chronic mammalian toxicity tests (Maphosa et al., 2010a)	Maphosa and Masika (2012)
Elephantorrhiza elephantina (Burch.) Skeels	R	DBA – *in vitro*	*H. contortus*	2.50	Negative – acute, sub-acute and chronic mammalian toxicity tests (Maphosa et al., 2010a)	Maphosa et al. (2010b)
Erythrophleum lasianthum Corbishley	L	DBA – *in vitro*	*C. elegans*	2.00	Unknown	McGaw et al. (2000)
Leucaena leucocephala (Lam.) de Wit	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – brine shrimp toxicity assay (McGaw and Eloff, 2005)	McGaw and Eloff (2005)
Medicago sativa L.	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – brine shrimp toxicity assay (McGaw and Eloff, 2005)	McGaw and Eloff (2005)

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Table 1 (*continued*)

Family Plant species	[a]Plant part(s)	[b]Assay method	Test organism(s)	[c]Concentration (mg/ml)	Safety findings? Method(s) and reference	Reference
Peltophorum africanum Sond.	L Bk R	DBA – *in vitro*	*H. contortus*	5.0 5.0 1.0	Negative – brine shrimp toxicity assay; and Vero monkey kidney cells cytotoxicity assay (Bizimenyera, 2007)	Bizimenyera et al. (2006b)
Peltophorum africanum Sond.	Rb	DBA – *in vivo*	*H. contortus* *T. colubriformis*	n/a	Negative – brine shrimp toxicity assay; and Vero monkey kidney cells cytotoxicity assay (Bizimenyera, 2007)	Bizimenyera et al. (2008)
Peltrophorum africanum Sond.	L Sb Rb	DBA – *in vitro*	*T. colubriformis*	0.619 (ED_{50}) 0.383 (ED_{50}) 0.280 (ED_{50})	Negative – brine shrimp toxicity assay; and Vero monkey kidney cells cytotoxicity assay (Bizimenyera, 2007)	Bizimenyera et al. (2006a)
Pseudarthria hookeri Wight & Arn.	L+R	DBA – *in vitro*	*S. haematobium*	50.0	Unknown	Sparg et al. (2000)
Pterocarpus angolensis DC.	Bk L	DBA – *in vitro*	*C. elegans*	1.00 2.00	Positive – brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2007)
Schotia brachypetala Sond.	Bk L	DBA – *in vitro*	*C. elegans*	1.00 2.00	Positive – brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2007)
Senna petersiana (Bolle) Lock.	L	CA – *in vitro*	*C. elegans*	0.52	Positive – Vero monkey kidney cell line cytotoxicity test (Tshikalange et al., 2005)	Aremu et al. (2010b)
Senna petersiana (Bolle) Lock.	—	DBA – *in vitro*	*S. haematobium*	6.25	Positive – Vero monkey kidney cell line cytotoxicity test (Tshikalange et al., 2005)	Sparg et al. (2000)
Hypoxidaceae						
Hypoxis colchicifolia Bak.	L C	CA – *in vitro*	*C. elegans*	0.13 2.08	Negative – Ames and VITOTOX® (Elgorashi et al., 2003); and positive – micronucleus test and comet assay (Taylor et al., 2003)	Aremu et al. (2010b)
Hypoxis hemerocallidea Fisch., C.A. Mey. & Avé-Lall.	L C	CA – *in vitro*	*C. elegans*	1.04 1.04	Negative – Ames test and VITOTOX® (Elgorashi et al., 2003; Reid et al., 2006); and positive – micronucleus test (Taylor et al., 2003)	Aremu et al. (2010b)
Icacinacea						
Cassinopsis illicifolia Hochst.	L Bk	CA – *in vitro*	*C. elegans*	0.033 0.016	Negative – Ames test (Okem et al., 2012)	Okem et al. (2012)
Apodytes dimidiate E. Mey. ex Arn.	L	DBA – *in vitro*	*C. elegans*	1.00	Negative – acute and sub-acute mammalian toxicity tests (Brackenbury et al., 1997)	McGaw et al. (2000)
Lamiaceae						
Leonotis leonurus (L.) R.Br.	L	DBA – *in vivo*	*Strongyle* *Trichuris* spp. *Eimeria* spp.	500 mg/kg	Positive/negative – acute, sub-acute and chronic mammalian toxicity tests (Maphosa et al., 2008)	Maphosa and Masika (2012)
Leonotis leonurus (L.) R.Br.	L	DBA – *in vitro*	*H. contortus*	1.25	Positive/negative – acute, sub-acute and chronic mammalian toxicity tests (Maphosa et al., 2008)	Maphosa et al. (2010b)
Leonotis leonurus (L.) R.Br.	L	DBA – *in vitro*	*C. elegans*	1.00	Positive/negative – acute, sub-acute and chronic mammalian toxicity tests (Maphosa et al., 2008)	McGaw et al. (2000)
Ocimum basilicum L.	L	CA – *in vitro*	*C. elegans*	0.26	Negative – acute and sub-acute mammal toxicity (Fandohan et al., 2008)	Aremu et al. (2010b)
Tetradenia riparia (Hochst.) Codd	L	CA – *in vitro*	*C. elegans*	0.004	Negative – Ames test (Okem et al., 2012)	Okem et al. (2012)
Malvaceae						
Gossypium herbaceum L.	L	DBA – *in vitro*	*C. elegans*	1.00	Negative – brine shrimp toxicity assay (McGaw and Eloff, 2005)	McGaw and Eloff (2005)
Meliaceae						
Ekebergia capensis Sparrm.	L	DBA – *in vitro*	*C. elegans*	2.00	Negative – Ames and VITOTOX® (Elgorashi et al., 2003; Reid et al., 2006); and toxic – micronucleus test (Taylor et al., 2003)	McGaw et al. (2000)
Melia azedarach L.	L	DBA – *in vitro*	*C. elegans*	1.00	Positive – review studies (Phua et al., 2008)	McGaw et al. (2000)
Trichilia emetic Vahl.	–	DBA – *in vitro*	*S. haematobium*	3.13	Negative – Ames and VITOTOX® (Elgorashi et al., 2003); Positive – Micronucleus test (Taylor et al., 2003)	Sparg et al. (2000)
Melianthaceae						
Melianthus comosus Vahl.	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – brine shrimp toxicity assay (McGaw and Eloff, 2005)	McGaw and Eloff (2005)
Melianthus major (L.)	L	DBA – *in vitro*	*C. elegans*	n/a	Positive – brine shrimp toxicity assay (McGaw and Eloff, 2005)	McGaw and Eloff (2005)
Myrsinaceae						
Maesa lanceolata Forssk.	L	DBA – *in vitro*	*C. elegans*	1.00	Unknown	McGaw et al. (2000)

Table 1 (*continued*)

Family Plant species	[a]Plant part(s)	[b]Assay method	Test organism(s)	[c]Concentration (mg/ml)	Safety findings? Method(s) and reference	Reference
Olacaceae						
Ximenia caffra Sond.	R	DBA – *in vitro*	*S. haematobium*	50.0	Positive – antiproliferative assay with three human cell lines (HeLa, HT29 and A431) (Kamuhabwa et al., 2000)	Sparg et al. (2000)
Pedaliaceae						
Dicerocaryum eriocarpum (Decne.) Abels	Wp	DBA – *in vitro*	*C. elegans*	1.00	Positive – brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2007)
Poaceae						
Sorghum bicolor (L.) Moench	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – brine shrimp toxicity assay (McGaw and Eloff, 2005)	McGaw and Eloff (2005)
Ranunculaceae						
Clematis brachiate Thunb.	L+S	DBA – *in vitro*	*S. haematobium*	25.0	Positive/negative – toxicity to mammalian liver, kidney and hematological parameters (Afolayan et al., 2009)	Sparg et al. (2000)
Rhamnaceae						
Berchemia zeyheri (Sond.) Grubov	Bk	DBA – *in vitro*	*C. elegans*	0.50	Positive – brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2007)
Ziziphus mucronata Willd.	Bk L	DBA – *in vitro*	*C. elegans*	n/a n/a	Positive – Ames and VITOTOX® (Elgorashi et al., 2003); micronucleus test and comet assay (Taylor et al., 2003); and brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2007)
Ziziphus mucronata Willd.	R	DBA – *in vitro*	*S. haematobium*	12.5	Positive – Ames and VITOTOX® (Elgorashi et al., 2003); micronucleus test and comet assay (Taylor et al., 2003); and brine shrimp toxicity assay (McGaw et al., 2007)	Sparg et al. (2000)
Rosaceae						
Leucosidea sericea Eckl. & Zeyh.	L S	CA – *in vitro*	*C. elegans*	0.26 2.08	Negative – Ames test (Aremu et al., 2011)	Aremu et al. (2010a)
Rubiaceae						
Canthium spinosum (Klotzsch ex Eckl. & Zeyh.) Kuntze	L	CA – *in vitro*	*C. elegans*	0.016	Negative – Ames test (Okem et al., 2012)	Okem et al. (2012)
Coddia rudis (E. Mey. ex Harv.) Verdc.	L	CA – *in vitro*	*C. elegans*	0.008	Negative – Ames test (Okem et al., 2012)	Okem et al. (2012)
Conostomium natalensis (Hochst.) Bremek.	L	CA – *in vitro*	*C. elegans*	0.270	Negative – Ames test (Okem et al., 2012)	Okem et al. (2012)
Lagynia lasiantha (Sond.) Bullock	L	CA – *in vitro*	*C. elegans*	0.065	Negative – Ames test (Okem et al., 2012)	Okem et al. (2012)
Rutaceae						
Clausena anisata (Willd) Hook. f. ex Benth.	L	DBA – *in vitro*	*C. elegans*	1.00	Unknown	McGaw et al. (2000)
Zanthoxylum capense (Thunb.) Harv.	L	DBA – *in vitro*	*C. elegans*	1.00	Negative – Ames test (Ndhlala et al., 2011)	McGaw et al. (2000)
Sapindaceae						
Hippobromus pauciflorus (L.f.) Radlk.	Ap	DBA – *in vitro*	*C. elegans*	0.50	Negative – brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2007)
Solanaceae						
Cestrum laevigatum Schltdl.	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – brine shrimp toxicity assay (McGaw and Eloff, 2005)	McGaw and Eloff (2005)
Nicotiana glauca Graham	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – brine shrimp toxicity assay (McGaw and Eloff, 2005)	McGaw and Eloff (2005)
Sterculiaceae						
Dombeya rotundifolia (Hochst.) Planch.	Ap	DBA – *in vitro*	*C. elegans*	1.00	Negative — Ames and VITOTOX® (Elgorashi et al., 2003); negative – micronucleus test (Taylor et al., 2003); and brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2007)
Thymelaeaceae						
Gnidia capitata L.f.	R	DBA – *in vitro*	*C. elegans*	1.00	Positive — brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2007)

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Table 1 (*continued*)

Family Plant species	[a]Plant part(s)	[b]Assay method	Test organism(s)	[c]Concentration (mg/ml)	Safety findings? Method(s) and reference	Reference
Ulmaceae						
Celtis africana N.L. Burm.	–	DBA – *in vitro*	*S. haematobium*	50.0	Negative – Ames and VITOTOX® (Elgorashi et al., 2003); and positive – micronucleus test (Taylor et al., 2003)	Sparg et al. (2000)
Trema orientalis (L.) Blume	Bk+wd	DBA – *in vitro*	*C. elegans*	1.00	Negative – Ames test (Hong and Lyu, 2011)	McGaw et al. (2000)
Urticaceae						
Pouzolzia mixta Solms-Laub.	L S	DBA – *in vitro*	*C. elegans*	n/a n/a	Negative – Ames and VITOTOX® (Elgorashi et al., 2003); micronucleus test and comet assay (Taylor et al., 2003); and positive – brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2007)
Verbenaceae						
Clerodendrum glabrum E. Mey.	L	DBA – *in vitro*	*C. elegans*	2.00	Unknown	McGaw et al. (2000)
Clerodendrum myricoides (Hochst.) Vatke.	L S	CA – *in vitro*	*C. elegans*	0.26 2.08	Positive – Ames test (Reid et al., 2006)	Aremu et al. (2010b)
Lantana camara L.	L	DBA – *in vitro*	*C. elegans*	n/a	Negative – brine shrimp toxicity assay (McGaw and Eloff, 2005)	McGaw and Eloff (2005)
Lantana rugosa Thunb.	L	DBA – *in vitro*	*C. elegans*	1.00	Positive – brine shrimp toxicity assay (McGaw and Eloff, 2005)	McGaw and Eloff (2005)
Lippia javanica (Burm.f.) Spreng	L+T	DBA – *in vitro*	*C. elegans*	1.00	Unknown	McGaw et al. (2000)
Vitaceae						
Cissus quadrangularis L.	S	DBA – *in vitro*	*C. elegans*	n/a	Positive – brine shrimp toxicity assay (McGaw et al., 2007)	McGaw et al. (2007)
Xanthorrhoeaceae						
Aloe ferox Mill.	L	DBA – *in vivo*	*Strongyle* *Trichuris* spp. *Eimeria* spp.	500 mg/kg	Negative – bacterial and mammalian cell genotoxicity assays (Andersen, 2007)	Maphosa and Masika (2012)
Aloe ferox Mill.	L	DBA – *in vitro*	*H. contortus*	20.0	Negative – bacterial and mammalian cell genotoxicity assays (Andersen, 2007)	Maphosa et al. (2010b)
Aloe marlothii Alwin Berger	L	DBA – *in vitro*	*C. elegans*	1.00	Unknown	McGaw et al. (2000)

[a] Plant part(s): Tb = tuber; Wd = wood; Wp = whole plant; Rm = rhizome; R = root; Rb = root bark; L = leaf; T = twig; Ap = aerial parts; Bk = bark; and C = corm.
[b] Assay method(s): DBA = Developmental and behavioral assay; and CA = colorimetric assay.
[c] Concentration (mg/ml): n/a = not active at highest concentration tested.

2012). Despite the efficacy and relative safety margins of the available chemotherapies, there is a growing concern about potential problems of acquired resistance as varying degrees of resistance in nematode populations in humans and livestock have been reported globally (Albonico et al., 2003; Jabbar et al., 2006). As further emphasized by Kaplan and Vidyashankar (2012), the 'inconvenient truth' is that the development of anthelmintic resistance will almost certainly eclipse the discovery of new classes of anthelmintics. In fact, recent studies have indicated that AADs are not active against relevant human STH such as *Necator americanus*, *Ancylostoma ceylanicum* and *Trichuris muris* (Tritten et al., 2011). In addition, the problem of re-infection after chemotherapy (Kirwan et al., 2009), adverse drug complications in certain groups of people such as hepatitis patients, pregnant and lactating women (Savioli et al., 2003) have been highlighted. For a long-time, the number of anthelmintic drug research projects in academic laboratories and pharmaceutical industries globally were limited (Geary et al., 1999a). Although more effort has been recently geared toward the search for new anthelmintic, the quantity of work and outputs is relatively small compared to other diseases such as cancer, HIV and malaria. This is partially due to limited financial gains to the multi-national pharmaceutical companies as the majority of people who suffer from helminthiasis are the poor (Smout et al., 2010). Researchers need to explore and validate novel solutions or to rediscover ancient knowledge for a more sustainable control of helminth diseases (Hoste and Torres-Acosta, 2011; Mrazek and Mossialos, 2003). The need to devote more efforts toward the discovery of new anthelmintics of plant origin has been strongly advocated (Athanasiadou et al., 2007; Waller et al., 2001). In fact, the importance of plants as one of the natural sources of drugs cannot be over-emphasized. An estimated 87% of the global medications used against cancer, microbial and parasitic infections are derived from natural products, particularly higher plants (Newman et al., 2003). Since antiquity, humans have used plants against ailments such as coughs, colds and parasitic infections; probably based on trial and error (Gurib-Fakim, 2006).

4. Potential anthelmintic medicinal plants

The use of plants and their extracts for the treatment of a variety of human and livestock gastro-intestinal parasites have been in existence for a long-time (Waller et al., 2001). Some of the earliest known medicinal anthelmintic plants include *Carica papaya*, *Ficus* species and *Ananas comosus*. Until recently, anti-parasitic activity of most medicinal plants was based on anecdotal evidence, but there is currently an increase in the number of scientific studies aiming to verify, validate, quantify and determine the safety of these plants (Athanasiadou et al., 2007). Globally, scientists have intensified efforts in the exploration of anthelmintics from natural products (Dornetshuber et al., 2009; Koné et al., 2011; Kotze et al., 2009; Muthee et al., 2011). On the positive side, anthelmintic compounds such as santonin and filicic acids were isolated from *Artemisia maritime* and *Dryopterix filix-mas*, respectively (Setzer and Vogler, 2006).

4.1. Contributions from South African researchers

In South Africa, medicinal plants have been used in the treatment of parasitic diseases of both man and livestock for centuries. The practice is still prevalent due to cultural reasons as well as the relatively high cost and inaccessibility of the existing chemotherapeutics (Clark et al., 1997). Evidence of the use of South African plants is well documented in the commonly-used literature (Hutchings et al., 1996; Watt and Breyer-Brandwijk, 1962) and researchers have investigated some of these South African plants for their anthelmintic efficacy (Table 1). We highlight 43 plant families consisting of 115 plant species that have been investigated for their anthelmintic property. The families included Combretaceae (21), Fabaceae (14), Asteraceae (7), Apocynaceae (7), Verbenaceae (5), Euphorbiaceae (4) and Rubiaceae (4) which accounted for more than 50% of the tested species. Based on the findings from cited studies, about 57% of the tested plant species exhibited various degrees of anthelmintic activity (Table 1). Based on the *in vitro* test systems, examples of South African plants with promising anthelmintic activity include extracts from *Markhamia obtusifolia* (Nchu et al., 2011), *Tulbaghia violacea* (McGaw et al., 2000), *Combretum molle* (Ademola and Eloff, 2010), *Hypoxis colchicifolia* (Aremu et al., 2010b), *Cassinopsis illicifolia*, *T. riparia*, and *Coddia rudis* (Okem et al., 2012). In addition, *Aloe ferox*, *Leonotis leonurus* and *Elephantorrhiza elephantina* (Maphosa and Masika, 2012) have been shown to be effective anthelmintic remedies under *in vivo* test systems.

Overall, the number of scientific reports in this field is small compared to antimicrobial investigations (Fig. 1). In South Africa, the continued neglect of anthelmintic studies is well pronounced with 8-fold more antimicrobial papers compared to anthelmintic papers published in South African Journal of Botany (Fig. 1). Even though current data were not based on a fully exhaustive and critical search of the entire database, it gives the general trend. There is a need to stimulate more interest and studies in this aspect of ethnopharmacology (Mrazek and Mossialos, 2003; Torres-Acosta et al., 2012). Particularly as helminthiasis is a serious problem to humans as well as livestock production and the increasing risk of drug resistance remains potentially detrimental (Patten et al., 2011). Despite the limited available studies, some pharmacological important compounds (*e.g.* butelinic and lupeol) exhibiting anthelmintic have been isolated from South African medicinal plants (Fig. 2). These compounds are also known for their diverse biological activities (Gallo and Sarachine, 2009; Yogeeswari and Sriram, 2005).

5. Anthelmintic activity assays and test-organisms

Helminths belong to a very large group of organisms that are evolutionary diverse in body structure, biology and parasitic mechanisms (Fennell et al., 2004; Geary and Thompson, 2001). It is thus imperative to ask questions that may mar or make the investigation. For instance, which test organism will be employed? What type of test system is required — *in vitro* or *in vivo*? In addition, important factors such as plant extracting methods and the effect of environmental and seasonal variations on the plant chemical composition, must be taken into consideration when evaluating the potential of medicinal plants for parasite control (Athanasiadou et al., 2007; Katiki et al., 2011). These critical questions must be answered based on the aims of the experiment, available equipment and financial resources. *In vivo* and *in vitro* bioassays, as well as a number of test organisms, have been employed to screen plants for anthelmintic activity (Table 1). Both test systems and the varieties of organisms employed have various limitations (Jabbar et al., 2006).

5.1. In vivo versus in vitro – benefits and drawbacks

As in the case with other pharmacological screening, it is paramount to always use the 'best practice' approaches to research into various disease states (Houghton et al., 2007). Most preliminary screening investigations of plants for anthelmintic activity utilize *in vitro* bioassays (Simpkin and Coles, 1981). As evident in Table 1, the majority (approximately 96%) of the studies in the current review were performed using *in vitro* systems. Generally, the main advantages of using *in vitro* bioassays include the low cost involved and rapid turnover that allow for large-scale screening of plants, as well as the low quantity of extracts required (Githiori et al., 2006; Whitfield, 1996). The weakness of *in vitro* tests is that researchers often extrapolate results from *in vitro* tests to claim *in vivo* activity and/or efficacy without taking into account vital factors such as absorption and bioavailability. Ideally, *in vitro* bioassays should be followed by *in vivo* tests when validating the anthelmintic activity of medicinal plants (Athanasiadou et al., 2007). However, it becomes relatively more expensive to screen plant extracts on a large scale using *in vivo* studies. As a result, many scientists are content with preliminary experiments culminating in the numerous papers based on *in vitro* screening (Table 1). Perhaps, better collaboration among local and international researchers as well as more financial support from the government and pharmaceutical companies would lessen the financial burdens. Inevitably, this could stimulate the feasibility of either scaling-up herbal mixture screening or the clinical trial of isolated compounds.

5.2. Commonly used test organisms

Numerous test models such as the free-living nematodes (*Caenorhabditis elegans*, *Rhabditis pseudoelongata*), earthworm (*Pheritima posthuma*), ascarids (*A. lumbricoides*), rodent nematode (*Heligmosomoides polygyrus*), trematode (*Schistosoma mansoni*), cestode (*Hymenolepis diminuta*) and trichostronylid (*H. contortus*) have been used in different studies (Githiori et al., 2006). As observed in this review (Table 1) however, *C. elegans* remains the most frequently used test organism. The organism has long been employed for anthelmintic screening with limited success especially, in terms of discovery of valuable new leads (Geary et al., 1999b). Apparently, it is regarded as the most suitable test organism for preliminary high-throughput *in vitro* screening for compounds with broad spectrum nematocidal activity (Geary and Thompson, 2001), being the best representative of a large phylum that contains several parasites (Bürglin et al., 1998). *C. elegans* belongs to the Order Rhabditida, which is closely associated with the Order Strongylida that contains the important trichostrongyle nematode parasites of ruminants, including *H. contortus* and *Trichostrongylus* spp. Furthermore, the sensitivity of *C. elegans* to most commercial anthelmintic drugs as well as the ease of culture growth and maintenance contributes to its widespread use for anthelmintic screenings (Katiki et al., 2011; Simpkin and Coles, 1981). *H. contortus* is also commonly used as it is known for causing serious problems in small ruminant production (Table 1). Despite the substantial benefits derived from the use of *C. elegans*, a number of limitations remain apparent (Geary and Thompson, 2001). There is no single test organism without limitations thus; two or more test organisms should be used for anthelmintic screening. Besides, anthelmintics with a broad spectrum efficacy are of more value and useful in the search for new compounds/drugs. For instance, AADs were tested and proved effective against a wide range of helminths such as *C. elegans* and *H. contortus* (Ducray et al., 2008; Kaminsky et al., 2008b) but ineffective against other STHs (Tritten et al., 2011).

5.3. Reappraisal of assay methods

Scientific evaluation or standards comparing the efficacy of plant extracts to commercial anthelmintics, as well as determining the level of anthelmintic activity are limited. As a result, there is high experimental variability that has led to an increased visible conflict of results on anthelmintic activity by plant extracts (Athanasiadou et al., 2007). The discovery of new anthelmintics relies to a certain extent on the availability of an effective, rapid screening assay to detect anthelmintic properties (Gnoula et al., 2007; Smout et al., 2010). Since the 1980s, the importance of automated screening involving the use of a micromotility meter has been recognized (Bennett and Pax, 1986; Folz et al., 1987). Despite the usefulness of the approach, the inherent limitations restrict its application to small scale studies (Das et al., 1988).

Researchers have employed different assays such as the larval arrested morphology assay (LAMA), larval development assay (LDA), larval motility assay (LMA), egg hatch test (EHT), adult development test (ADT), motility and colorimetric assays (Jabbar et al., 2006; Kopp et al., 2008) to evaluate anthelmintic activity. These various tests/assays can be partially divided into two major categories: i) developmental and behavioral assays (DBA) and ii) colorimetric assays (CA). In DBA, these anthelmintic studies require measuring the survival and/or reproductive potential of the worms after incubating with the test extracts for a specific time. The efficacy of the tested extracts/compounds is often measured based on the worms' viability (reproduction responses) or mobility (behavior responses) after the incubation period (McGaw et al., 2000). Basically, the worms are stimulated (if necessary) and classified as either alive or dead/paralyzed. The efficacy is reported based on the relative number of death and paralysis due to the extract/drug compared to the controls. However, DBA are generally subjective, often unreliable, time consuming and laborious (Abdulla et al., 2009; Von Samson-Himmelstjerna et al., 2009). In addition, it is always difficult to distinguish between the paralyzed and dead worms (Gnoula et al., 2007). Thus, a visual, qualitative and quantitative worm viability assessment was developed (James and Davey, 2007).

In the CA methods, the test organisms are incubated with extracts or conventional drugs for specific duration. Thereafter, the metabolic activity is utilized as the measure of the test organism viability after the addition of an indicator compound, *e.g.* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide-formazan (MTT-formazan). Gnoula et al. (2007) also described a similar method with the use of a different indicator, 5(6)-carboxyfluorescein diacetate (CFDA) which fluoresced dead worms. Generally, CA provides a simple method for viability testing in potential anthelmintic plant extracts against *C. elegans* with the advantage of rapid evaluation. Several South African medicinal plants were screened with a modified CA method (Aremu et al., 2010a; Okem et al., 2012). The use of *p*-iodonitrotetrazolium chloride (INT) instead of MTT-formazan worked well due to the better stability of INT. However, the use of CA is strictly only useful for *in vitro* evaluation and seems to have some inherent limitations. For instance, the use of response to indicators is likely to lead to loss of some potential anthelmintic extracts and compounds with moderate activity.

Recently, the successful application of a novel cell monitoring device (xCELLigence, Roche Inc.) system for anthelmintic screening was demonstrated (Silbereisen et al., 2011; Smout et al., 2010). The technique assessed the real-time anti-parasite efficacy of synthetic anthelmintics on eggs, larvae and adults in a fully automated, label-free manner. It is a simple and objective high-throughput assay for anthelmintics screening as well as enabling resistance diagnosis by real-time monitoring of parasite motility. The method is postulated to be valuable for the majority of helminth species and developmental stages where EHA or motility is used as a measure of worm viability. As recently demonstrated however, xCELLigence system may only be useful in the presence of adult hookworms (Tritten et al., 2012b). Despite the shortcoming of xCELLigence system, the application of this cutting-edge technology may be an invaluable tool for exploring the rich South African botanicals. However, the high cost could possibly hinder the availability of this cutting-edge technology to researchers in developing countries.

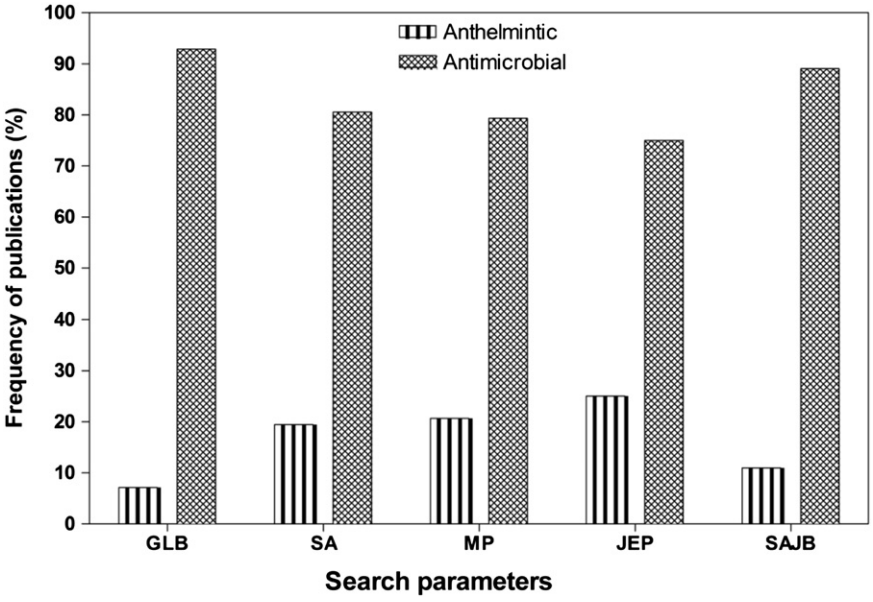
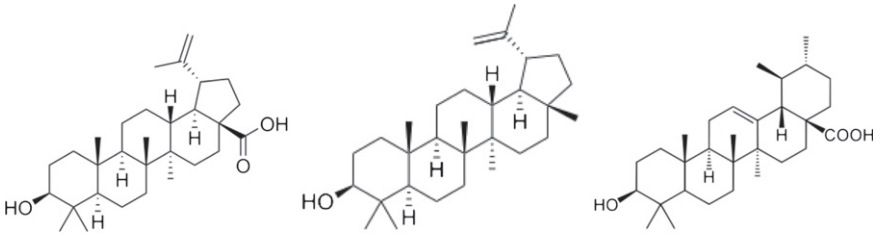


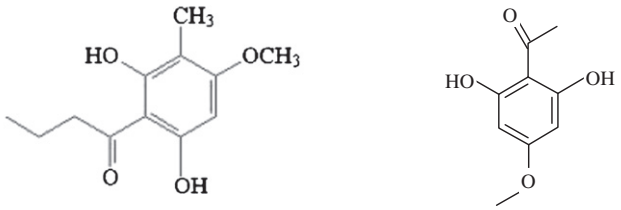
Fig. 1. Elsevier-derived data comparing the frequency of antimicrobial and anthelmintic articles using different search parameters. GLB = global; SA = South Africa; MP = medicinal plants; JEP = Journal of Ethnopharmacology and SAJB = South African Journal of Botany (only 2006–2012 available online). Data shown are only meant to give an indication of the general trend rather than for comprehensive analysis purposes.

6. Safety of medicinal plants used as anthelmintics

A series of detailed systematic tests are basic and stringent prerequisites before pharmaceutical companies release any anthelmintic drug into the market. Thus, information on the efficacy, mode of action, pharmacokinetic parameters and potential environmental side-effects as well as the direct and indirect toxicities of the drug is known. Conversely, similar information on plant extracts or herbal products are limited or non-existent (Hoste and Torres-Acosta, 2011). The widely held accepted belief and assumption are that natural products are safe, based on their long-term usage by humans and livestock (Verschaeve and Van Staden, 2008). Nevertheless, recent investigations have revealed that some plants do have mutagenic and perhaps toxic effects (Elgorashi et al., 2003; Barlow and Schlatter, 2010). This raises concern about the



Betulinic acid **1** Lupeol **2** Ursolic acid **3**



Aspidinol **4** Desaspidinol **5**

Fig. 2. Examples of anthelmintic compounds isolated from South Africa medicinal plants. 1, 2 and 3 = *Curtisia dentate* (Shai et al., 2009); and 4 and 5 = *Leucosidea sericea* (Bosman et al., 2004).

potential mutagenic health hazards resulting from the long-term use of such medicinal plants. Even though medicinal plants have benefits such as low cost, availability and acceptability, their safety and toxicity need to be evaluated and documented (Hoste and Torres-Acosta, 2011; Street et al., 2008).

In order to distinguish a clearly toxic effect from pharmacological efficacy, it is appropriate to incorporate toxicity and/or mutagenic tests when screening medicinal plants. Due to diverse mechanisms responsible for toxic and mutagenic effects, the importance of choosing the most appropriate and relevant safety evaluation method cannot be overemphasized. Generally, the Ames test is recommended for initial screening for mutagenic potential due to its long history of usage as well as the proven high degree of reproducibility of results and sensitivity (Mortelmans and Zeiger, 2000). The Ames test was successfully used to screen a wide range of South African medicinal plant extracts (Verschaeve and Van Staden, 2008) and commercial herbal mixtures (Ndhlala et al., 2010). The inclusion of S9 metabolic activation has been based on evidence that some mutagenic plant extracts or compounds only exhibit their mutagenicity after undergoing activation and transformation by enzymatic and metabolic processes (Elgorashi et al., 2003; Reid et al., 2006). Other commonly used safety evaluation methods include the micronucleus test, comet and brine shrimp assays with their inherent pros and cons. For example, the brine shrimp assay is a simple, inexpensive technique for detecting cytotoxic potentials; however, it remains ineffective when such compounds required metabolic activation to become toxic (McGaw et al., 2007). It is recommended that *in vitro* safety evaluation should be followed by *in vivo* experiments for confirmatory results.

6.1. Evaluation of the safety of South African plants used as anthelmintics

Table 1 highlights the safety evaluation of common South African medicinal plants with anthelmintic potential. In addition to their promising anthelmintic activity, *H. colchicifolia* (Elgorashi et al., 2003; Taylor et al., 2003) and *A. dimiata* (Brackenbury et al., 1997) were shown to be safe whereas *D. eriocarpum*, *B. zeyheri*, *Gnidia capitata* (McGaw et al., 2007), *A. calamus* (Padmaja et al., 2002), *Senna petersiana* (Tshikalange et al., 2005) and *Clerodendrum myricoides* (Reid et al., 2006) are potentially unsafe due to their toxic or mutagenic inducing effects. Furthermore, in other plant species such as *Acacia karoo* (Adedapo et al., 2008), *L. leonurus* (Maphosa et al., 2008) and *Clematis brachiata* (Afolayan et al., 2009), the toxicity has been demonstrated to be dependent on the concentration of the extracts. Unfortunately, limited scientific data exist on the safety of other species such as *Acokanthera oppositifolia*, *Mondia whitei* and *Berkheya speciosa*. Thus, caution must be exercised when administering these plant extracts as anthelmintic remedies for both humans and livestock.

7. Concluding remarks and future perspectives

Helminth infections affect a large proportion of the population, especially those living in informal and rural settlements as well as their livestock. There is also a growing resistance to the available chemotherapeutics with frequent cases of reinfection occurring. As with any class of drugs used against infective pathogens, development of resistance to new AADs (latest class of anthelmintics) remains a major concern (Prichard and Geary, 2008). Medicinal plants provide mixtures of many chemical compounds possessing multiple biological activities. In South Africa, the vast number of medicinal plants and the traditional medicinal system remains poorly explored for anthelmintics. Thus, appropriate screening and evaluation of more medicinal plants with evidence of anthelmintic activity would offer possible alternative remedies that are sustainable. Besides validating the use of traditional medicines by providing a homecare herbal remedy against helminth infection, it could also serve as a preliminary phase in discovering leads for new anthelmintics.

However, researchers have continued to neglect anthelmintic investigation compared to other pharmacological properties such as antimicrobial, anticancer and anti-HIV activities. *In vitro* screening is important in validating the traditional use of medicinal plants as well as for providing leads in the search for new active principles. It could be a means of rapidly screening for potential anthelmintic activity in medicinal plant extracts and providing evidence for the possible mechanism(s) of the active compound(s). More so, the number of isolated bioactive compounds demonstrating anthelmintic activity is low.

Majority of the researchers have used the simplistic *in vitro* assays. It is essential to remember that noteworthy pharmacological activity identified in *in vitro* test does not always directly confirm that a plant extract is an effective medicine, or a suitable candidate for drug development. In view of the differences between *in vitro* and *in vivo* environments, active extracts should be subjected to *in vivo* investigation with the use of different parasitic test organisms. It is high time that we step out of the comfort zone by advancing the preliminary studies to *in vivo* systems, and ultimately clinical trials. Based on the inadequacy of current screening methods, we need to critically review and improve them for better efficiency. Finally, attempts at the isolation and identification of bioactive compounds as well as toxicity testing to determine the safety of the medicinal plants should be pursued rigorously. We recommend more collaboration among researchers both locally and internationally, the South African government should also increase the funding and incentives for scientists focusing on neglected diseases affecting the poor. In addition, an increase in the number of quality studies focusing on South African plants used as anthelmintics would certainly contribute positively to this expanding ethnopharmacological field.

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References

Abdulla, M.H., Ruelas, D.S., Wolff, B., Snedecor, J., Lim, K.C., Xu, F., Renslo, A.R., Williams, J., McKerrow, J.H., Caffrey, C.R., 2009. Drug discovery for schistosomiasis: hit and lead compounds identified in a library of known drugs by medium-throughput phenotypic screening. Public Library of Science Neglected Tropical Diseases 3, e478.

Adedapo, A.A., Sofidiya, M.O., Masika, P.J., Afolayan, A.J., 2008. Safety evaluations of the aqueous extract of *Acacia karroo* stem bark in rats and mice. Record of Natural Products 2, 128–134.

Ademola, I.O., Eloff, J.N., 2010. *In vitro* anthelmintic activity of *Combretum molle* (R. Br. ex G. Don) (Combretaceae) against *Haemonchus contortus* ova and larvae. Veterinary Parasitology 169, 198–203.

Afolayan, A.J., Yakubu, M.T., Appidi, J.R., Mostafa, M., 2009. Toxicological implications of aqueous extract of *Clematis brachiata* Thunb. leaves in male Wistar rats. African Journal of Pharmacy and Pharmacology 3, 531–538.

Albonico, M., Bickle, Q., Ramsan, M., Montresor, A., Savioli, L., Taylor, M., 2003. Efficacy of mebendazole and levamisole alone or in combination against intestinal nematode infections after repeated targeted mebendazole treatment in Zanzibar. Bulletin of the World Health Organization 81, 343–352.

Amabeoku, G.J., Kabatende, J., 2012. Antinociceptive and anti-inflammatory activities of leaf methanol extract of *Cotyledon orbiculata* L. (Crassulaceae). Advances in Pharmacological Sciences, http://dx.doi.org/10.1155/2012/862625.

Andersen, F.A., 2007. Final report on the safety assessment of *Aloe andongensis* extract, *Aloe andongensis* leaf juice, *Aloe arborescens* leaf extract, *Aloe arborescens* leaf juice, *Aloe arborescens* leaf protoplasts, *Aloe barbadensis* flower extract, *Aloe barbadensis* leaf, *Aloe barbadensis* leaf extract, *Aloe barbadensis* leaf juice, *Aloe barbadensis* leaf polysaccharides, *Aloe barbadensis* leaf water, *Aloe ferox* leaf extract, *Aloe ferox* leaf juice, *Aloe ferox* leaf juice extract. International Journal of Toxicology 26, 1–50.

Appleton, C.C., Maurihungirire, M., Gouws, E., 1999. The distribution of helminth infections along the coastal plain of KwaZulu-Natal Province, South Africa. Annals of Tropical Medicine and Parasitology 93, 859–868.

Aremu, A.O., Fawole, O.A., Chukwujekwu, J.C., Light, M.E., Finnie, J.F., Van Staden, J., 2010a. *In vitro* antimicrobial, anthelmintic and cyclooxygenase-inhibitory activities and phytochemical analysis of *Leucosidea sericea*. Journal of Ethnopharmacology 131, 22–27.

Aremu, A.O., Ndhlala, A.R., Fawole, O.A., Light, M.E., Finnie, J.F., Van Staden, J., 2010b. *In vitro* pharmacological evaluation and phenolic content of ten South African medicinal plants used as anthelmintics. South African Journal of Botany 76, 558–566.

Aremu, A.O., Amoo, S.O., Ndhlala, A.R., Finnie, J.F., Van Staden, J., 2011. Antioxidant activity, acetylcholinesterase inhibition, iridoid content and mutagenic evaluation of *Leucosidea sericea*. Food and Chemical Toxicology 49, 1122–1128.

Athanasiadou, S., Githiori, J., Kyriazakis, I., 2007. Medicinal plants for helminth parasite control: facts and fiction. Animal 1, 1392–1400.

Barlow, S., Schlatter, J., 2010. Risk assessment of carcinogens in food. Toxicology and Applied Pharmacology 243, 180–190.

Behnke, J.M., Buttle, D.J., Stepek, G., Lowe, A.E., Duce, I.R., 2008. Developing novel anthelmintics from plant cysteine proteinases. Parasite and Vectors 1, 29, http://dx.doi.org/10.1186/1756-3305-1-29.

Bennett, J.L., Pax, R.A., 1986. Micromotility meter: an instrument designed to evaluate the action of drugs on motility of larval and adult nematodes. Parasitology 93, 341–346.

Bizimenyera, E.S., 2007. The potential role of antibacterial, antioxidant and antiparasitic activity of *Peltophorum africanum* Sond. (Fabaceae) extracts in ethnoveterinary. PhD Thesis, Phytomedicine Programme, Department of Paraclinical Sciences, University of Pretoria. Pretoria, South Africa.

Bizimenyera, E.S., Githiori, J.B., Eloff, J.N., Swan, G.E., 2006a. *In vitro* activity of *Peltophorum africanum* Sond. (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode *Trichostrongylus colubriformis*. Veterinary Parasitology 142, 336–343.

Bizimenyera, E.S., Githiori, J.B., Swan, G.E., Eloff, J.N., 2006b. *In vitro* ovicidal and larvicidal activity of the leaf, bark and root extracts of *Peltophorum africanum* Sond. (Fabaceae) on *Haemonchus contortus*. Journal of Animal and Veterinary Advances 5, 608–614.

Bizimenyera, E.S., Meyer, S., Naidoo, V., Eloff, J.N., Swan, G.E., 2008. Efficacy of *Peltophorum africanum* Sond. (Fabaceae) extracts on *Haemonchus contortus* and *Trichostrongylus colubriformis* in sheep. Journal of Animal and Veterinary Advances 7, 364–371.

Booth, M., Bundy, D.A.P., Albonico, M., Chwaya, H.M., Alawi, K.S., Savioli, L., 1998. Associations among multiple geohelminth species infections in school-children from Pemba Island. Parasitology 116, 85–93.

Borkow, G., Bentwich, Z., 2006. HIV and helminth co-infection: is deworming necessary? Parasitology Immunology 28, 605–612.

Bosman, A.A., Combrinck, S., Roux-Van der Merwe, R., Botha, B.M., McCrindle, R.I., 2004. Isolation of an anthelmintic compound from *Leucosidea sericea*. South African Journal of Botany 70, 509–511.

Brackenbury, T.D., Appleton, C.C., Thurman, G., 1997. Mammal toxicity assessment of the plant molluscicide, *Apodytes dimidiata* (Icacinaceae), in South Africa. Acta Tropica 65, 155–162.

Brooker, S., Bethony, J., Hotez, P.J., 2004. Human hookworm infection in the 21st century. Advances in Parasitology 58, 197–288.

Bürglin, T.R., Lobos, E., Blaxter, M.L., 1998. *Caenorhabditis elegans* as a model for parasitic nematodes. International Journal for Parasitology 28, 395–411.

Chabala, J.C., Mrozik, H., Tolman, R.L., Eskola, P., Lusi, A., Peterson, L.H., Woods, M.F., Fisher, M.H., Campbell, W.C., 1980. Ivermectin, a new broad-spectrum antiparasitic agent. Journal of Medicinal Chemistry 23, 1134–1136.

Clark, T.E., Appleton, C.C., Kvalsvig, J.D., 1997. Schistosomiasis and the use of indigenous plant molluscicides: a rural South African perspective. Acta Tropica 66, 93–107.

Das, A.K., Bhattacharya, S., Chatterjee, G.K., Chaudhuri, S.K., 1988. Simple micromotility recorder for rapid screening of potentially anthelmintic compounds. Journal of Pharmacological Methods 20, 323–327.

Dornetshuber, R., Kamyar, M.R., Rawnduzi, P., Baburin, I., Kouri, K., Pilz, E., Hornbogen, T., Zocher, R., Berger, W., Lemmens-Gruber, R., 2009. Effects of the anthelmintic drug PF1022A on mammalian tissue and cells. Biochemical Pharmacology 77, 1437–1444.

Ducray, P., Gauvry, N., Pautrat, F., Goebel, T., Fruechtel, J., Desaules, Y., Weber, S.S., Bouvier, J., Wagner, T., Froelich, O., Kaminsky, R., 2008. Discovery of amino-acetonitrile derivatives, a new class of synthetic anthelmintic compounds. Bioorganic & Medicinal Chemistry Letters 18, 2935–2938.

Elgorashi, E.E., Taylor, J.L.S., Maes, A., Van Staden, J., De Kimpe, N., Verschaeve, L., 2003. Screening of medicinal plants used in South African traditional medicine for genotoxic effects. Toxicology Letters 143, 195–207.

Elias, D., Mengistu, G., Akuffo, H., Britton, S., 2006. Are intestinal helminthes risk factors for developing active tuberculosis? Tropical Medicine & International Health 11, 551–558.

Elliott, D.E., Summers, R.W., Weinstock, J.V., 2007. Helminths as governors of immune-mediated inflammation. International Journal for Parasitology 37, 457–464.

Evans, A.C., Du Preez, L., Maziya, S.P., Van der Merwe, C.A., Schutte, C.H.J., 1987. Observations on the helminth infections in black pupils of the Eastern Transvaal Lowveld of South Africa. South Africa Journal of Epidemiology and Infections 2, 7–14.

Fandohan, P., Gnonlonfin, B., Laleye, A., Gbenou, J.D., Darboux, R., Moudachirou, M., 2008. Toxicity and gastric tolerance of essential oils from *Cymbopogon citratus*, *Ocimum gratissimum* and *Ocimum basilicum* in Wistar rats. Food and Chemical Toxicology 46, 2493–2497.

Fennell, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M., Van Staden, J., 2004. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. Journal of Ethnopharmacology 94, 205–217.

Fincham, J.E., Evans, A.C., Woodrof, C.W., Seager, J.R., Benade, A.J.S., Appleton, C.C., 1996. Feed the children, not the parasites — an essential part of primary health care in South Africa. South African Medical Journal 86, 647–649.

Fincham, J.E., Markus, M.B., Adams, V.J., 2003. Could control of soil-transmitted helminthic infection influence the HIV/AIDS pandemic. Acta Tropica 86, 315–333.

Folz, S.D., Pax, R.A., Thomas, E.M., Bennett, J.L., Lee, B.L., Conder, G.A., 1987. Detecting *in vitro* anthelmintic effects with a micromotility meter. Veterinary Parasitology 24, 241–250.

Gallo, M.B.C., Sarachine, M.J., 2009. Biological activities of lupeol. International Journal of Biomedical and Pharmaceutical Sciences 3, 46–66.

Geary, T.G., 2005. Ivermectin 20 years on: maturation of a wonder drug. Trends in Parasitology 21, 530–532.

Geary, T.G., Thompson, D.P., 2001. *Caenorhabditis elegans*: how good a model for veterinary parasites? Veterinary Parasitology 101, 371–386.

Geary, T.G., Sangster, N.C., Thompson, D.P., 1999a. Frontiers in anthelmintic pharmacology. Veterinary Parasitology 84, 275–295.

Geary, T.G., Thompson, D.P., Klein, R.D., 1999b. Mechanism-based screening: discovery of the next generation of anthelmintics depends upon more basic research. International Journal of Parasitology 29, 105–112.

Geary, T.G., Woo, K., McCarthy, J.S., Mackenzie, C.D., Horton, J., Prichard, R.K., De Silva, N.R., Olliaro, P.L., Lazdins-Helds, J.K., Engels, D.A., Bundy, D.A., 2010. Unresolved issues in anthelmintic pharmacology for helminthiases of humans. International Journal for Parasitology 40, 1–13.

Githiori, J.B., Athanasiadou, S., Thamsborg, S.M., 2006. Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. Veterinary Parasitology 139, 308–320.

Gnoula, C., Guissou, I., Dubois, J., Duez, P., 2007. 5(6)-Carboxyfluorescein diacetate as an indicator of *Caenorhabditis elegans* viability for the development of an *in vitro* anthelmintic drug assay. Talanta 71, 1886–1892.

Gqaleni, N., Moodley, I., Kruger, H., Ntuli, A., McLeod, H., 2007. Traditional and complementary medicine. In: Harrison, S., Bhana, R., Ntuli, A. (Eds.), South African Health Review. Health Systems Trust, Pretoria, South Africa.

Gurib-Fakim, A., 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine 27, 1–93.

Hirschowitz, R., Orkin, M., 1997. Inequality in South Africa: findings from the 1994 October household survey. Social Indicators Research 41, 119–136.

Hong, C.E., Lyu, S.Y., 2011. Genotoxicity detection of five medicinal plants in Nigeria. The Journal of Toxicological Sciences 36, 87–93.

Horak, G.E., Ursula, P.R.E., 2004. Parasites of domestic and wild animals in South Africa. XLV. Helminths of dairy calves on dry-land Kikuyu grass pastures in the Eastern Cape Province. The Onderstepoort Journal of Veterinary Research 71, 291–306.

Hoste, H., Torres-Acosta, J.F.J., 2011. Non chemical control of helminths in ruminants: adapting solutions for changing worms in a changing world. Veterinary Parasitology 180, 144–154.

Hotez, P.J., Molyneux, D.H., Fenwick, A., Ottesen, E., Ehrlich, S.S., Sachs, J.D., 2006. Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, malaria. Public Library of Science Medicine 3, e102, http://dx.doi.org/10.1371/journal.pmed.0030102.

Hotez, P.J., Molyneux, D.H., Fenwick, A., Kumaresan, J., Sachs, S.E., Sachs, J.D., Savioli, L., 2007. Control of neglected tropical diseases. The New England Journal of Medicine 357, 1018–1027.

Hotez, P.J., Fenwick, A., Savioli, L., Molyneux, D.H., 2009. Rescuing the bottom billion through control of neglected tropical diseases. Lancet 373, 1570–1575.

Houghton, P.J., Howes, M.J., Lee, C.C., Steventon, G., 2007. Uses and abuses of *in vitro* tests in ethnopharmacology: visualizing an elephant. Journal of Ethnopharmacology 110, 391–400.

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A., 1996. Zulu medicinal plants. An Inventory. University of Natal Press, Pietermaritzburg, South Africa.

Jabbar, A., Iqbal, Z., Kerboeuf, D., Muhammad, G., Khan, M.N., Afaq, M., 2006. Anthelmintic resistance: the state of play revisited. Life Sciences 79, 2413–2431.

Jäger, A.K., 2005. Is traditional medicine better off 25 years later? Journal of Ethnopharmacology 100, 3–4.

James, C.E., Davey, M.W., 2007. A rapid colorimetric assay for the quantitation of the viability of free-living larvae of nematodes *in vitro*. Parasitology Research 101, 975–980.

Jinabhai, C.C., Taylor, M., Coutsoudis, A., Coovadia, H.M., Tomkins, A.M., Sullivan, K.R., 2001. Epidemiology of helminth infections: implications for parasite control programmes, a South African perspective. Public Health Nutrition 4, 1211–1219.

Kaminsky, R., Gauvry, N., Schorderet Weber, S., Skripsky, T., Bouvier, J., Wenger, A., Schroeder, F., Desaules, Y., Hotz, R., Goebel, T., Hosking, B., Pautrat, F., Wieland-Berghausen, S., Ducray, P., 2008a. Identification of the amino-acetonitrile derivative monepantel (AAD 1566) as a new anthelmintic drug development candidate. Parasitology Research 103, 931–939.

Kaminsky, R., Ducray, P., Jung, M., Clover, R., Rufener, L., Bouvier, J., Weber, S.S., Wenger, A., Wieland-Berghausen, S., Goebel, T., Gauvry, N., Pautrat, F., Skripsky, T., Froelich, O., Komoin-Oka, C., Westlund, B., Sluder, A., Maser, P., 2008b. A new class of anthelmintics effective against drug-resistant nematodes. Nature 452, 176–180.

Kamuhabwa, A., Nshimo, C., De Witte, P., 2000. Cytotoxicity of some medicinal plant extracts used in Tanzanian traditional medicine. Journal of Ethnopharmacology 70, 143–149.

Kaplan, R.M., Vidyashankar, A.N., 2012. An inconvenient truth: global worming and anthelmintic resistance. Veterinary Parasitology, http://dx.doi.org/10.1016/j.vetpar.2011.11.048.

Katiki, L.M., Ferreira, J.F.S., Zajac, A.M., Masler, C., Lindsay, D.S., Chagas, A.C.S., Amarante, A.F.T., 2011. *Caenorhabditis elegans* as a model to screen plant extracts and compounds as natural anthelmintics for veterinary use. Veterinary Parasitology 182, 264–268.

Kirwan, P., Asaolu, S., Molloy, S., Abiona, T., Jackson, A., Holland, C., 2009. Patterns of soil-transmitted helminth infection and impact of four-monthly albendazole treatments in preschool children from semi-urban communities in Nigeria: a double-blind placebo-controlled randomised trial. BioMed Central Infectious Diseases 9, http://dx.doi.org/10.1186/1471-2334-9-20.

Koné, W., Vargas, M., Keiser, J., 2011. Anthelmintic activity of medicinal plants used in Côte d'Ivoire for treating parasitic diseases. Parasitology Research, http://dx.doi.org/10.1007/s00436-011-2771-z.

Kopp, S.R., Coleman, G.T., McCarthy, J.S., Kotze, A.C., 2008. Application of *in vitro* anthelmintic sensitivity assays to canine parasitology: detecting resistance to pyrantel in *Ancylostoma caninum*. Veterinary Parasitology 152, 284–293.

Kotze, A.C., O'Grady, J., Emms, J., Toovey, A.F., Hughes, S., Jessop, P., Bennell, M., Vercoe, P.E., Revell, D.K., 2009. Exploring the anthelmintic properties of Australian native shrubs with respect to their potential role in livestock grazing systems. Parasitology 136, 1065–1080.

Mabaso, M.L.H., Appleton, C.C., Hughes, J.C., Gouws, E., 2004. Hookworm (*Necator americanus*) transmission in inland areas of sandy soils in KwaZulu-Natal, South Africa. Tropical Medicine & International Health 9, 471–476.

Magen, E., Borkow, G., Bentwich, Z., Mishal, J., Scharf, S., 2005. Can worms defend our hearts? Chronic helminthic infections may attenuate the development of cardiovascular diseases. Medical Hypotheses 64, 904–909.

Maphosa, V., Masika, P., 2012. *In vivo* validation of *Aloe ferox* (Mill). *Elephantorrhiza elephantina* Bruch. Skeels. and *Leonotis leonurus* (L) R. BR as potential anthelminthics and antiprotozoals against mixed infections of gastrointestinal nematodes in goats. Parasitology Research 110, 103–108.

Maphosa, V., Masika, P., Adedapo, A., 2008. Safety evaluation of the aqueous extract of *Leonotis leonurus* shoots in rats. Human and Experimental Toxicology 27, 837–843.

Maphosa, V., Masika, P.J., Moyo, B., 2010a. Toxicity evaluation of the aqueous extract of the rhizome of *Elephantorrhiza elephantina* (Burch.) Skeels. (Fabaceae), in rats. Food and Chemical Toxicology 48, 196–201.

Maphosa, V., Masika, P.J., Bizimenyera, E.S., Eloff, J.N., 2010b. *In-vitro* anthelminthic activity of crude aqueous extracts of *Aloe ferox*, *Leonotis leonurus* and *Elephantorrhiza elephantina* against *Haemonchus contortus*. Tropical Animal Health and Production 42, 301–307.

McGaw, L.J., Eloff, J.N., 2005. Screening of sixteen poisonous plants for antibacterial, anthelmintic and cytotoxic activity in vitro. South African Journal of Botany 71, 302–306.

McGaw, L.J., Jäger, A.K., Van Staden, J., 2000. Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. Journal of Ethnopharmacology 72, 247–263.

McGaw, L.J., Rabe, T., Sparg, S.G., Jäger, A.K., Eloff, J.N., Van Staden, J., 2001. An investigation on the biological activity of *Combretum* species. Journal of Ethnopharmacology 75, 45–50.

McGaw, L.J., Van der Merwe, D., Eloff, J.N., 2007. *In vitro* anthelmintic, antibacterial and cytotoxic effects of extracts from plants used in South African ethnoveterinary medicine. The Veterinary Journal 173, 366–372.

McKellar, Q.A., Jackson, F., 2004. Veterinary anthelmintics: old and new. Trends in Parasitology 20, 456–461.

Messias, J.B., Caraciolo, M.C.M., De Oliveira, I.M., Montarroyos, U.R., Bastos, I.V.G.A., Guerra, M.d.O., Souza, I.A., 2010. Evaluation hematological and biochemical parameters of rats in the second thirds of the gestation submitted to the action of the extract methanol of *Cereus jamacaru* DC., Cactaceae. Revista Brasileira de Farmacognosia 20, 478–483.

Mkhize-Kwitshana, Z., Taylor, M., Jooste, P., Mabaso, M., Walzl, G., 2011. The influence of different helminth infection phenotypes on immune

responses against HIV in co-infected adults in South Africa. BioMed Central Infectious Diseases 11, 273.
Mortelmans, K., Zeiger, E., 2000. The Ames Salmonella/microsome mutagenicity assay. Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis 455, 29–60.
Mrazek, M.F., Mossialos, E., 2003. Stimulating pharmaceutical research and development for neglected diseases. Health Policy 64, 75–88.
Murray, P.R., Rosenthal, K.S., Kobayashi, G.S., Pfaller, M.A., 1998. Medical Microbiology, 3rd ed. Mosby Inc., Missouri, USA.
Muthee, J.K., Gakuya, D.W., Mbaria, J.M., Kareru, P.G., Mulei, C.M., Njonge, F.K., 2011. Ethnobotanical study of anthelmintic and other medicinal plants traditionally used in Loitoktok district of Kenya. Journal of Ethnopharmacology 135, 15–21.
Nchu, F., Githiori, J.B., McGaw, L.J., Eloff, J.N., 2011. Anthelmintic and cytotoxic activities of extracts of *Markhamia obtusifolia* Sprague (Bignoniaceae). Veterinary Parasitology 183, 184–188.
Ndhlala, A.R., Anthonissen, R., Stafford, G.I., Finnie, J.F., Verschaeve, L., Van Staden, J., 2010. *In vitro* cytotoxic and mutagenic evaluation of thirteen commercial herbal mixtures sold in KwaZulu-Natal, South Africa. South African Journal of Botany 76, 132–138.
Ndhlala, A.R., Finnie, J.F., Van Staden, J., 2011. Plant composition, pharmacological properties and mutagenic evaluation of a commercial Zulu herbal mixture: *Imbiza ephuzwato*. Journal of Ethnopharmacology 133, 663–674.
Newman, D.J., Cragg, G.M., Snader, K.M., 2003. Natural products as sources of new drugs over the period 1981–2002. Journal of Natural Products 66, 1022–1037.
Okem, A., Finnie, J.F., Van Staden, J., 2012. Pharmacological, genotoxic and phytochemical properties of selected South African medicinal plants used in treating stomach-related ailments. Journal of Ethnopharmacology 139, 712–720.
Padmaja, R., Arun, P.C., Prashanth, D., Deepak, M., Amit, A., Anjana, M., 2002. Brine shrimp lethality bioassay of selected Indian medicinal plants. Fitoterapia 73, 508–510.
Patten, T., Good, B., Hanrahan, J., Mulcahy, G., De Waal, T., 2011. Gastrointestinal nematode control practices on lowland sheep farms in Ireland with reference to selection for anthelmintic resistance. Irish Veterinary Journal 64, 4.
Phua, D.H., Tsai, W.J., Ger, J., Deng, J.F., Yang, C.C., 2008. Human *Melia azedarach* poisoning. Clinical Toxicology 46, 1067–1070.
Prichard, R.K., Geary, T.G., 2008. Drug discovery: fresh hope to can the worms. Nature 452, 157–158.
Prichard, R.K., Basáñez, M.G., Boatin, B.A., McCarthy, J.S., García, H.H., Yang, G.J., Sripa, B., Lustigman, S., 2012. A research agenda for helminth diseases of humans: intervention for control and elimination. Public Library of Science Neglected Tropical Diseases 6, e1549.
Rang, H.P., Dale, M.M., Ritter, J.M., Moore, P.K., 2003. Anthelmintic drugs, In: Rang, H.P., Dale, M.M., Ritter, J.M., Moore, P.K. (Eds.), Pharmacology, 5th ed. Churchill Livingstone, London, UK, pp. 687–692.
Reid, K.A., Maes, J., Maes, A., Van Staden, J., De Kimpe, N., Mulholland, D.A., Verschaeve, L., 2006. Evaluation of the mutagenic and antimutagenic effects of South African plants. Journal of Ethnopharmacology 106, 44–50.
Saathoff, E., Olsen, A., Kvalsvig, J.D., Appleton, C.C., 2004. Patterns of geohelminth infection, impact of albendazole treatment and re-infection after treatment in schoolchildren from rural KwaZulu-Natal/South-Africa. BioMed Central Infectious Diseases 4, 27.
Savioli, L., Bundy, D., Tomkins, A., 1992. Intestinal parasitic infections: a soluble public health problem. Transactions of the Royal Society of Tropical Medicine and Hygiene 86, 353–354.
Savioli, L., Crompton, D.W.T., Neira, M., 2003. Use of anthelminthic drugs during pregnancy. American Journal of Obstetrics and Gynecology 188, 5–6.
Schutte, C.H.J., Fripp, P.J., Evans, A.C., 1995. An assessment of the schistosomiasis situation in the republic of South Africa. Southern African Journal of Epidermiology and Infection 10, 37–43.
Setzer, W.N., Vogler, B., 2006. Bioassays for activity, In: Cseke, L.J., Kirakosyan, A., Kaufman, P.B., Warber, S.L., Duke, J.A., Brielmann, H.L. (Eds.), Natural Products from Plants, 2nd ed. Taylor and Francis Group, New York, USA, pp. 389–414.
Shai, L.J., Bizimenyera, E.S., Bagla, V., McGaw, L.J., Eloff, J.N., 2009. *Curtisia dentata* (Cornaceae) leaf extracts and isolated compounds inhibit motility of parasitic and free-living nematodes. The Onderstepoort Journal of Veterinary Research 76, 249–256.
Silbereisen, A., Tritten, L., Keiser, J., 2011. Exploration of novel *in vitro* assays to study drugs against *Trichuris* spp. Journal of Microbiological Methods 87, 169–175.
Simpkin, K.G., Coles, G.C., 1981. The use of *Caenorhabditis elegans* for anthelmintic screening. Journal of Chemical Technology and Biotechnology 31, 66–69.
Sinniah, B., 1984. Intestinal protozoan and helminth infections and control of soil-transmitted helminths in Malay school children. Public Health 98, 152–156.
Smout, M.J., Kotze, A.C., McCarthy, J.S., Loukas, A., 2010. A novel high throughput assay for anthelmintic drug screening and resistance diagnosis by real-time monitoring of parasite motility. Public Library of Science Neglected Tropical Diseases 4, e885.
Sparg, S.G., Van Staden, J., Jäger, A.K., 2000. Efficiency of traditionally used South African plants against schistosomiasis. Journal of Ethnopharmacology 73, 209–214.
Stepek, G., Buttle, D.J., Duce, I.R., Behnke, J.M., 2006. Human gastrointestinal nematode infections: are new control methods required? International Journal of Experimental Pathology 87, 325–341.
Street, R.A., Stirk, W.A., Van Staden, J., 2008. South African traditional medicinal plant trade — challenges in regulating quality, safety and efficacy. Journal of Ethnopharmacology 119, 705–710.
Taylor, J.L.S., Elgorashi, E.E., Maes, A., Van Gorp, U., De Kimpe, N., Van Staden, J., Verschaeve, L., 2003. Investigating the safety of plants used in South African traditional medicine: testing for genotoxicity in the micronucleus and alkaline comet assays. Environmental and Molecular Mutagenesis 42, 144–154.
Torres-Acosta, J.F.J., Molento, M., Mendoza de Gives, P., 2012. Research and implementation of novel approaches for the control of nematode parasites in Latin America and the Caribbean: is there sufficient incentive for a greater extension effort? Veterinary Parasitology, http://dx.doi.org/10.1016/j.vetpar.2011.11.053.
Tritten, L., Silbereisen, A., Keiser, J., 2011. *In vitro* and *in vivo* efficacy of Monepantel (AAD 1566) against laboratory models of human intestinal nematode infections. Public Library of Science Neglected Tropical Diseases 5, e1457.
Tritten, L., Silbereisen, A., Keiser, J., 2012a. Nitazoxanide: *in vitro* and *in vivo* drug effects against *Trichuris muris* and *Ancylostoma ceylanicum*, alone or in combination. International Journal for Parasitology: Drugs and Drug Resistance 2, 98–105.
Tritten, L., Braissant, O., Keiser, J., 2012b. Comparison of novel and existing tools for studying drug sensitivity against the hookworm *Ancylostoma ceylanicum in vitro*. Parasitology 139, 348–357.
Tritten, L., Nwosu, U., Vargas, M., Keiser, J., 2012c. *In vitro* and *in vivo* efficacy of tribendimidine and its metabolites alone and in combination against the hookworms *Heligmosomoides bakeri* and *Ancylostoma ceylanicum*. Acta Tropica 122, 101–107.
Tshikalange, T.E., Meyer, J.J.M., Hussein, A.A., 2005. Antimicrobial activity, toxicity and the isolation of a bioactive compound from plants used to treat sexually transmitted diseases. Journal of Ethnopharmacology 96, 515–519.
Van de Venter, M., Roux, S., Bungu, L.C., Louw, J., Crouch, N.R., Grace, O.M., Maharaj, V., Pillay, P., Sewnarian, P., Bhagwandin, N., Folb, P., 2008. Antidiabetic screening and scoring of 11 plants traditionally used in South Africa. Journal of Ethnopharmacology 119, 81–86.
Van Wyk, J.A., Malan, F.S., Gerber, H., Alves, R., 1987. Two field strains of *Haemonchus contortus* resistant to rafoxanide. The Onderstepoort Journal of Veterinary Research 54, 143–146.
Van Wyk, J.A., Stenson, M.O., Van der Merwe, J.S., Vorster, R.J., Viljoen, P.G., 1999. Anthelmintic resistance in South Africa: surveys indicate an extremely serious situation in sheep and goat farming. The Onderstepoort Journal of Veterinary Research 66, 273–284.
Vatta, A.F., Kandu-Lelo, C., Ademola, I.O., Eloff, J.N., 2011. Direct anthelmintic effects of *Cereus jamacaru* (Cactaceae) on trichostrongylid nematodes of sheep: *in vivo* studies. Veterinary Parasitology 180, 279–286.
Verschaeve, L., Van Staden, J., 2008. Mutagenic and antimutagenic properties of extracts from South African traditional medicinal plants. Journal of Ethnopharmacology 119, 575–587.

Von Samson-Himmelstjerna, G., Walsh, T.K., Donnan, A.A., Carrière, S., Jackson, F., Skuce, P.J., Rohn, K., Wolstenholme, A.J., 2009. Molecular detection of benzimidazole resistance in *Haemonchus contortus* using real-time PCR and pyrosequencing. Parasitology 136, 349–358.

Waller, P.J., Bernes, G., Thamsborg, S.M., Sukura, A., Richter, S.H., Ingebrigtsen, K., Hoglund, J., 2001. Plants as de-worming agents of livestock in the Nordic countries: historical perspective, popular beliefs and prospects for the future. Acta Veterinaria Scandinavica 42, 31–44.

Wang, L.J., Cao, Y., Shi, H.N., 2008. Helminth infections and intestinal inflammation. World Journal of Gastroenterology 14, 5125–5132.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed. Livingstone, London, UK.

Whitfield, P.J., 1996. Novel anthelmintic compounds and molluscicides from medicinal plants. Transactions of the Royal Society of Tropical Medicine and Hygiene 90, 596–600.

WHO, 1987. Public Health Significance of Intestinal Parasitic Infections. WHO — World Health Organization.

Wolday, D., Mayaan, S., Mariam, Z.G., Berhe, N., Seboxa, T., Britton, S., Galai, N., Landay, A., Bentwich, Z., 2002. Treatment of intestinal worms is associated with decreased HIV plasma viral load. JAIDS Journal of Acquired Immune Deficiency Syndromes 31, 56–62.

Yogeeswari, P., Sriram, D., 2005. Betulinic acid and its derivatives: a review on their biological properties. Current Medicinal Chemistry 12, 657–666.

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